THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Copyright © 1968 by The Williams & Wilkins Co.

Vol. 50, No. 4
Printed in U.S.A.

THE HEALING OF SKIN WOUNDS IN PRIMATES

II. THE PROLIFERATION OF EPIDERMAL CELL MELANOCYTES*

LUIGI GIACOMETTI, PH.D. AND WILLIAM MONTAGNA, PH.D.

In this short report we will show that epidermal melanocytes are capable of proliferating in injured skin and that this response is closely related to that of surrounding keratinocytes. Previously, melanocytes in mitosis have been observed by Bizzozero (1) in man, Billingham (2) in guinea pigs, and by others in a variety of mammals. We have earlier shown (3) that epidermal melanocytes take up tritiated thymidine after the skin is injured, but at that time did not see them actually in mitosis.

MATERIALS AND METHODS

Four young healthy male rhesus monkeys (*Macaca mulatta*) weighing between 3 and 4 kg were used. Under aseptic conditions, 3 cuts 10 mm long and 4 mm deep were made with a blade on the scalp of each animal. This region was chosen because, unlike the skin elsewhere, it contains an appreciable population of melanotic epidermal melanocytes (4). The wounds were not sutured nor was a dressing applied. Biopsy specimens of the wounded skin were taken daily for 12 days and fixed for 24 hours in Bouin's fluid. Phencyclidine hydrochloride (Sernylan®), 0.5 mg/kg body weight, injected parenterally, was used to establish basal narcosis at the time of the skin biopsy. The specimens were oriented so that the wound edge was cut transversely. Sections 6 μ thick were stained in Harris hematoxylin and eosin. All biopsy specimens were obtained at the same hour of the day (11:00 A.M.). The monkeys were kept in individual restraining chairs in a room where a constant temperature of 21° ± 2° C was maintained. They were fed a standard commercial monkey chow with water ad libitum.

OBSERVATIONS

We will mention here only those details of the process in the healing of these small wounds that are relevant to proliferation of melanocytes. Bleeding was slight immediately after wounding, and leukocytic infiltration occurred after 3–4 hours. Within 20 hours there was a generalized edema and vascular dilation in the dermis and a layer of polymorphonuclear cells separated the uninjured dermis from the wound. Epidermal proliferation and migration began on the 2nd day and continued through the 6th day, when "tongues" of epidermis from the opposite sides of the wound came together and fused to close the gap. The epidermal sheets then grew down into the upper reaches of the dermis. After 10 to 20 days there was an increased proliferation of fibroblasts and collagen fibers became stratified in horizontal layers.

We found melanocytes in the advancing epithelium from the 2nd to the 5th day after wounding but they were behind its forward edge. Most of them contained a small amount of melanin in the perikaryon and their dendrites were short or absent. Figures 1, 2, 3, 4, 5 and 6 show melanocytes undergoing typical mitotic division 6 to 10 days after wounding. Since only melanocytes contain some pigment granules in the cytoplasm it was evident that during the early phases of the healing of the wounds, some melanocytes were present in advancing epithelial sheets but none were present at any time in the advancing edge of the epithelial "tongues". Proliferation of melanocytes always coincided with the epithelial closure of the incisional gap. These cells, then, became active when the surrounding epidermal cells displayed hyperplasia, an increase in DNA synthesizing cells (5), and intensified mitotic activity (6). In the normal epidermis of these animals we have rarely found dividing melanocytes.

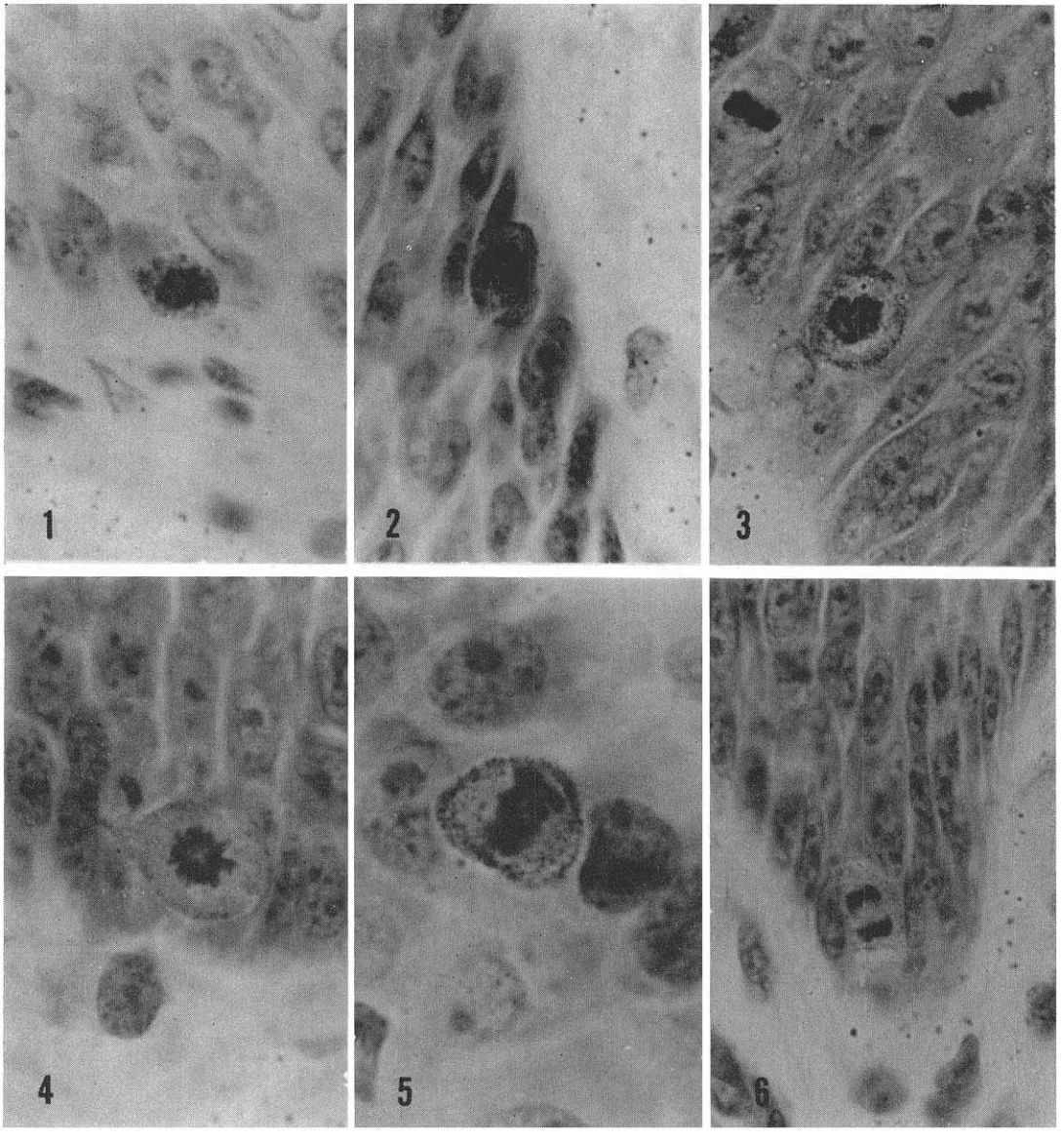
COMMENTS

Since the reader may not be familiar with the melanocytes resident in the epidermis of rhesus monkeys, we describe them briefly for

* From the Department of Cutaneous Biology, Oregon Regional Primate Research Center, Beaverton, Oregon.

Publication No. 289 from the Oregon Regional Primate Research Center; supported in part by Grant FR 00163 of the National Institutes of Health and by funds from Grant AM 08445, Biology of the Skin of Man and Other Primates; also supported by the Revlon Research Center, Inc., New York.

Accepted for publication September 20, 1967.



FIGS. 1 and 2.—Late prophase. The chromosomes are coiling up; the melanin granules are dispersed in the cytoplasm.

FIG. 3.—Prometaphase. The nuclear envelope has disappeared and the chromosomes are coiled into compact gyres.

FIG. 4.—Metaphase. The chromosomes are arranged equatorially. Note a short spike laden with melanin, probably a retracted dendrite.

FIG. 5.—Early anaphase. The chromosomes begin to pull apart. Note the peripheral localization of melanin granules.

FIG. 6.—Late anaphase. The chromosomes are near the poles and the cell is beginning to pinch in two.

the purpose of orientation. Except for that of the nose, ears, and friction surfaces, the epidermis of the rhesus monkey is mostly free of melanotic melanocytes (8). In the hairy skin a few melanin containing dopa-positive dendritic melanocytes are normally found only on the scalp (4). That the epidermis does contain a full complement of melanocytes, therefore,

can be demonstrated by irradiating the skin with ultraviolet light (7). After such treatment melanin containing, dopa-positive dendritic cells appear in the basal epidermal layer but the melanin granules that they produce remain within their dendritic processes, there being no evidence that the keratinocytes become the receptor cells of pigment. Thus, these cells are different from the melanocytes of most other species, and the separation of keratinocytes from melanocytes here is facilitated by the presence of melanin granules in the former.

An account of the healing of cutaneous wounds in the rhesus monkey has already been given by Giacometti (6). In this study we add only information on the biological properties of the melanocytes which, to our knowledge, have been studied only in our laboratory (4, 7, 8). We emphasize that in the hairy skin of the rhesus monkey, these cells are melanotic only on the scalp (4). They are further unique in that when they become melanotic after stimulation with ultraviolet light the melanin they form remains in their own cytoplasm (7). The melanocytes, therefore, are quickly separated from keratinocytes, which are free of pigment. Since these melanocytes do not begin to divide until the 6th day after wounding, when the incisional gap has been bridged by the epithelial tongues, we believe that there is a functional partnership between the melanocytes and the malpighian system. The physiologic conditions and integrity of the epidermis appear to determine the behavior of the melanocytes in it. The major purpose of this presentation has been to give a graphic documentation of the mitotic activity of epidermal melanocytes in rhesus monkeys.

SUMMARY

Small wounds were made in the scalp of rhesus monkeys and biopsy specimens were excised daily. From the second to the fifth day after wounding, epidermal melanocytes, mostly round and containing a few pigment granules, were found in the advancing epithelium but not at the wound margin. From 6 to 10 days after wounding, melanocytes were found in all stages of mitosis. Damage to the skin, then, provokes first a migration and then proliferation of both systems, keratinocytes and the accompanying melanocytes. Thus, the behavior of the melanocytes may depend upon the physiological condition and integrity of the surrounding epidermis.

REFERENCES

1. Bizzozero, E.: Sulle cellule cromatofore e di Langerhans nella pelle. Archivie per le science mediche, *30:* 611, 1910.
2. Billingham, R. E.: Dendritic cells. J. Anat., *8:* 93, 1948.
3. Giacometti, L. and Allegra, F.: The effect of wounding upon the uptake of ^{3}H-Thymidine by guinea pig epidermal melanocytes. *Advances in Biology of Skin. Vol. 8.* The Pigmentary System. Eds., Montagna, W. and Hu, F., Pergamon Press, Oxford, England, 1967.
4. Machida, H. and Perkins, E.: The distribution of melanotic melanocytes in the skin of subhuman primates. *Advances in Biology of Skin. Vol. 8.* The Pigmentary System. Eds., Montagna, W. and Hu, F., Pergamon Press, Oxford, England, 1967.
5. Gelfand, S.: Initiation of mitosis in relation to the cell division cycle. Exp. Cell Res., *26:* 395, 1962.
6. Giacometti, L.: The healing of skin wounds in primates. I. The kinetics of cell proliferation. J. Invest. Derm., *48:* 133, 1967.
7. Yun, J. S. and Montagna, W.: The melanocytes in the epidermis of the rhesus monkey (*Macaca mulatta*). Anat. Rec., *154:* 161, 1966.
8. Montagna, W., Yun, J. S., and Machida, H.: The skin of primates. XVIII. The skin of the rhesus monkey (*Macaca mulatta*). Am. J. Phys. Anthrop., *22:* 307, 1964.